

Appl. No. 09/760,379
Amdt. Dated July 14, 2004
Reply to Office Action of May 14, 2004

REMARKS

Claims 1, 5-9, 11, 14, and 15 were pending in the present application. By this Amendment, Applicants have amended claim 9, which is now written in independent form. The present Amendment introduces no new matter, and thus, its entry is respectfully requested. Upon its entry, claims 1, 5-9, 11, 14, and 15 will remain pending and under examination.

The May 14, 2004 Final Office Action

Rejections withdrawn:

The Examiner has withdrawn the previous rejections under 35 U.S.C. §102 as a result of Applicants' previous response.

In response, Applicants acknowledge and appreciate the withdrawal of these rejections.

Examiner's new rejection under 35 U.S.C. §103

The Examiner rejected claims 1, 5-8, and 11 under 35 U.S.C. §103(a) as being unpatentable over WO 98/12717, Anselmann, et al. According to the Examiner, Anselmann, et al. teaches the preparation of monodisperse magnetic particles 50-1500 nm in size. Specifically, a spherical silicon dioxide core is coated with magnetic particles of Fe_3O_4 having a maximum particle size of 60 nm and having a cover layer of silicon dioxide, which has free or hydrated O or OH groups on the surface (page 3, paragraphs 4 and 6; page 7, paragraph 2). According to the Examiner, the surface of the magnetic particle can be modified by a silanizing agent (formula (I)), having functional groups (e.g., a hydrophobic group of alkyl C1-C6 and a hydrophilic group of COOR) by which reversible binding of the material to be separated from the magnetic

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particles can be achieved (page 6, paragraph 4-page 7, paragraph 1). The Examiner acknowledged that Anselmann, et al. does not specifically disclose the isolation of protein from aqueous solution, but asserted that the reference does refer to magnetic particles used to isolate nucleic acid, biotin and biotinylated proteins from aqueous solutions, and does provide the isolation of DNA, wherein DNA can be extracted from agarose gel by mixing with magnetic particles in a buffer, removing the remaining liquid from the particles, washing the magnetic particles with a buffer, and drying and resuspending the particles. The Examiner indicated that the DNA is eluted with a buffer solution which can be separated from the magnetic particles in a magnetic field (page 7, paragraphs 3-5). The Examiner has taken the position that at the time the invention was made, it would have been obvious to a person of ordinary skill in the art to isolate proteins using the procedure for isolating DNA as indicated in Anselmann, et al. because the reference indicates that the magnetic particles modified as above with a silanizing agent of formula (I) are to be used in the isolation of biotinylated proteins "among others" from aqueous solutions. (claim 5 of Anselmann).

In response, Applicants respectfully travers the Examiner's rejection. At the outset, Applicants disagree with the Examiner's characterization that Anselmann refers to the isolation of biotinylated proteins "among others" from aqueous solutions. It appears to Applicants that the only suggestion of protein isolation is that of biotin and biotinylated proteins, and no others.

The claims of the present invention refer, *inter alia*, to methods in which the solid phase used in the process comprises a combination of hydrophobic groups and hydrophilic groups on

its surface. The hydrophobic groups serve to bind the proteinaceous material. The proteinaceous material is bound reversibly and unspecifically to the hydrophobic groups located on the surface of the solid phase. The hydrophilic groups on the surface of this solid phase prevent the solid phase from sticking together in an aqueous environment. The combination of hydrophilic and hydrophobic groups provides particles that do not agglutinate in aqueous solution, but are still capable of binding proteinaceous material.

Anselmann, et al. teaches the preparation of monodisperse magnetic particles, in which a spherical silicon dioxide core is coated with magnetic particles Fe_3O_4 having a cover layer of silicon dioxide which has free or hydrated OH groups on the surface, to which the functional groups can be attached. These functional groups are coated to the silicon dioxide layer which surrounds the core of the particle, by suitable silanizing agents (page 6, 3rd paragraph).

Consequently, Anselmann et al. discloses three different kinds of particles:

The first one is one in which the cover layer of the particle core is formed of silicon dioxide with its free and hydrated OH groups on the surface. These particles can be used for the isolation of nucleic acids (page 7, 4th paragraph).

In the second group of particles, the surface of the particle is completely covered by covalently bound groups resulting from silanizing agents (page 6, 4th paragraph). These groups can also be used for the isolation of nucleic acids (page 7, 4th paragraph).

In the third group of particles streptavidin is covalently coupled to the groups constituting the outermost layer of particles of group two. These streptavidin-coated particles can be used for the isolation and purification of biotinylated proteins (page 8, 2nd paragraph).

Nucleic acids can be isolated from aqueous solutions using immobilized SiOH groups in a high salt environment and at neutral or low pH, followed by an elution under low salt conditions. For the isolation of nucleic acids a modification of the SiO₂ surface of the magnetic core particle using a silanizing agent is not necessary, as nucleic acids are directly bonded to the free and hydrated OH groups of the silicon dioxide layer surrounding the core of the particle (page 7, 4th paragraph).

Because of the polyanionic character of DNA, with always one negative charge per nucleotide, the isolation of DNA is almost trivial.

As proteins are composed of more than 20 different amino acids, their overall composition can be quite different. This results in different characteristics, for example, regarding overall charge composition, solubility, hydrophobicity, etc. Therefore, a general procedure for isolating DNA out of an aqueous solution cannot be applied for isolating proteins. Therefore, Anselmann, et al. teaches the use of additional streptavidin tags. In a further reaction step, these tags are additionally coated to the functional groups attached to the SiO₂ core cover layer as described above for the second group of particles (page 8, 2nd paragraph). To be bound by streptavidin tags, proteins of an aqueous solution have to be biotinylated in an additional reaction step. The binding of biotinylated proteins to the streptavidin tag is highly specific. It is

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the prior art to isolate biotinylated proteins using streptavidin tags in terms of a highly effective purification of single recombinant proteins out of a proteinaceous solution.

In sharp contrast to the prior art, the particles of the present invention achieve unspecific binding, i.e. binding which covers all proteinaceous materials, not only particular proteins modified by a tag recognition sequence. Therefore, for example, neither an additional protein modifying reaction step nor an expensive biotinylating agent is needed. The teaching of Anselmann et al., the highly specific isolation or purification, respectively, of a biotinylated protein, therefore, leads away from the object of the present invention, namely a protein isolation process which unspecifically catches all proteins of a proteinaceous solution based on unspecific reversible protein binding.

In sum, the essential aspect of the method of the invention, namely the mixing of hydrophilic and hydrophobic groups for unspecific binding of all proteins contained in a proteinaceous solution, respectively, on the surface of a solid phase, is neither described nor suggested by the cited reference. Thus, one of only ordinary skill in the art would not reasonably expect, based on the Anselmann reference, to be able to successfully isolate proteinaceous material bound unspecifically to hydrophobic groups on the surface of a solid phase, as claimed in the present invention. Accordingly, the claimed invention is not rendered obvious by the cited document, and thus, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1, 5-8, and 11 under 35 U.S.C. §103(a) .

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Examiner's claim objection

The Examiner objected to claims 9, 14, and 15 as being dependent on a rejected base claim. The Examiner has indicated that these claims would be allowable if rewritten in independent form.

Applicants acknowledge and appreciate the Examiner's allowance of the subject matter contained in claims 9, 14, and 15. Without conceding the correctness of the Examiner's rejection of the base claim, Applicants nevertheless have now rewritten claim 9 in independent form.

In view of the above amendments and remarks, it is believed that the claims satisfy the requirements of the patent statutes and fully address the Examiner's concerns as set forth in the May 14, 2004, Final Office Action. Reconsideration of the instant application and early notice of allowance therefore are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

No fee is believed due in connection with the filing of this Amendment. If, however, any fee is required, authorization is hereby given to charge such fee to Deposit Account No. 02-2135.

Respectfully submitted,



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